The time- and calcium-dependent association of recombinant human copine I or annexin A1 with supported lipid bilayers composed of 25% brain PS and 75% DOPC was monitored by atomic force microscopy. Neither protein bound to featureless areas of the bilayer but both rapidly bound to small domains that appeared to be 0.5 to 0.8 nm lower than the rest of the bilayer. These domains may be enriched in PS and/or have a more disordered lipid structure. Copine I assembled into a reticular pattern made of 40nm linear elements that appeared to be one or two molecules high. In vivo such copine arrays might form a scaffold for the assembly of signalling proteins bound by copine I. Annexin A1 did not form ordered structures but appeared to promote the growth of the domains of lowered height to which it was bound. These enlarged domains created by annexin A1 provided binding sites for copine I when it was added subsequently. Therefore, in vivo, annexin A1 might recruit C2 domain-containing proteins like copine to membranes by modulating membrane structure.

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**Depletion Of Intracellular Cholesterol Disrupts Carbachol But Not PTH-mediated $Ca^{2+}$ Signals In HEK293 Cells**

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In HEK cells stably expressing receptors for type 1 parathyroid hormone (PTH), PTH increases the sensitivity of $IP_3$ receptors ($IP_3R$) to $IP_3$ via a cAMP-dependent mechanism, thereby potentiating $Ca^{2+}$ signals evoked by muscarinic $M_3$ receptors that stimulate $IP_3$ formation. The effect of PTH results from cAMP binding directly to a low-affinity site on either the $IP_3R$ itself or a protein tightly associated with it. cAMP appears to pass directly from AC to $IP_3R$ via an association we have termed an AC-$IP_3R$ junction, formed selectively by AC6 and $IP_3R2$. Here we show disruption of cholesterol-rich lipid microdomains differentially disrupts $M_3R$ signaling in HEK cells.

In the absence of extracellular $Ca^{2+}$, stimulation of HEK cells with a maximal concentration of carbachol (CCh, 1mM) caused an increase in $[Ca^{2+}]_i$ of 249 ± 33nM which returned to basal within 60–70s. Subsequent addition of PTH, in the continued presence of CCh, evoked further concentration-dependent ($EC_{50}$ = 59 ± 15nM) increases in $[Ca^{2+}]_i$. Treatment with the cholesterol-depleting agent MβCD (2h; 22°C) caused an 86 ± 3% decrease in the response to CCh whilst having no significant effect on the response to PTH. Single-cell imaging revealed that treatment with MβCD caused a 30% decrease in the number of cells responding to CCh and a 45% decrease in amplitude of the $Ca^{2+}$ signal in cells that did respond. Filipin staining of free cholesterol confirmed that MβCD caused depletion of cellular cholesterol.

Depletion of intracellular cholesterol with MβCD disrupts CCh but not PTH signalling in HEK293 cells. We hypothesise this may be due to the existence of either two different $M_3R$ populations in the PM or differential distributions of $IP_3R$ isoforms in the ER.

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**Increased Store-Operated $Ca^{2+}$ Entry in Skeletal Muscle with Knockdown of Calsequestrin**

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Malignant hyperthermia (MH) is a life-threatening syndrome triggered by volatile anesthetics, in which uncontrolled elevation of myoplasmic $Ca^{2+}$ leads to hypercontracture of skeletal muscle and elevation of body temperature. Our recent study showed that azumolene, an analog of dantrolene used to treat MH, inhibits a component of store-operated $Ca^{2+}$ entry (SOCE) coupled to activation of the ryanodine receptor in skeletal muscle (*JBC* **281**: 33477, 2006). Given our previous observation that overexpression of calsequestrin-1 (CSQ1) suppressed SOCE in skeletal muscle (*JBC* **278**: 3286, 2003), here we tested the hypothesis that reduced CSQ1 expression would enhance an azumolene-sensitive SOCE in this tissue. A shRNA probe specific for CSQ1 (*JBC* **281**: 15772, 2006) was introduced into flexor digitorum brevis (FDB) muscles of living mice using electroporation. Individual transfected FDB muscle fibers labeled with a fluorescent marker were isolated for SOCE measurements using Mn-quenching of Fura-2 fluorescence. At room temperature (20–22°C), SOCE induced by caffeine/ryanodine was significantly enhanced in CSQ1-knockdown muscle fibers (in $10^{-4}$ $\Delta F_{360}$/s, 9.36 ± 1.31) compared to those transfected with control (4.71 ± 1.29, $p<0.05$). Pre-incubation with azumolene (20 μM) completely inhibited the elevated SOCE detected in CSQ1-knockdown fibers (1.26 ± 0.38, $p<0.01$). To prevent muscle contraction, we used *N*-benzyl-*p*-toluene sulfonamide (BTS, 40 μM), a specific myosin II inhibitor. When temperature of the bathing solution was increased to 40°C, muscle fibers with knockdown of CSQ1 displayed a significant elevation in cytosolic $Ca^{2+}$ over that seen in control fibers. Thus reduced CSQ1 expression is likely coupled to elevation of cytosolic $Ca^{2+}$ due to increased SOCE function at higher temperatures. These results suggest that elevated SOCE activity in skeletal muscle may be linked to the pathophysiology of MH and the heat-sensitivity of MH-susceptible animals.

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**Role of the Ryanodine Receptor/Calcium Release Channel in Beta-adrenergic Receptor Blocker Treatment of Heart Failure**

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To explore the role of protein kinase A (PKA) phosphorylation of the cardiac ryanodine receptor (RyR2)/calcium release channel in the treatment of heart failure (HF) using beta-adrenergic receptor blockers (beta-blockers) we generated a knock-in mouse with aspartic acid replacing serine at residue 2808 in RyR2 (RyR2-S2808D). This mutation mimics constitutive PKA hyperphosphorylation of RyR2, a condition that occurs during HF. RyR2-S2808D+/+ mice developed an age-dependent cardiomyopathy characterized by moderate cardiac dysfunction and mild left ventricular dilatation indicating that PKA hyperphosphorylation of RyR2 alone can cause cardiac dysfunction. Following myocardial infarction (MI), RyR2-S2808D+/+ mice exhibited increased mortality compared to WT littermates. Treatment with the rycal S107, a 1,4-benzothiazepine derivative that inhibits PKA hyperphosphorylation-induced depletion of calstabin2 from the RyR2 complex, for 4 weeks significantly reduced HF progression in WT and RyR2-S2808D+/+ mice, confirming the important role of calstabin2 binding to RyR2 in preventing HF progression. In contrast, following MI, treatment with the beta-adrenergic receptor blocker (beta-blocker) metoprolol improved cardiac function in WT but not in RyR2-S2808D+/+ mice, indicating the important role of inhibition of PKA hyperphosphorylation of RyR2 as a key mechanism underlying the beneficial effects of beta-blockers in HF. Taken together, these data show that chronic RyR2 PKA hyperphosphorylation alone can cause a cardiomyopathy, preventing calstabin2 depletion from the RyR2 macromolecular complex can inhibit HF progression, and PKA phosphorylation of RyR2 is an important determinant of the therpaeutic efficacy of beta-blocker therapy of HF.

**591-Pos Board B470**

**Structural Basis for Calcium Sensing by GCaMP2**

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Genetically encoded Ca2+ indicators are important tools that enable the measurement of Ca2+ dynamics in a physiologically relevant context. GCaMP2, one of the most robust indicators, is a circularly permutated EGFP (cpEGFP)/M13/Calmodulin (CaM) fusion protein, that has been successfully used for studying Ca2+ fluxes in vivo in the heart and vasculature of transgenic mice. Here we describe crystal structures of bright and dim states of GCaMP2 that reveal a sophisticated molecular mechanism for Ca2+ sensing. In the bright state, CaM stabilizes the fluorophore in an ionized state similar to that observed in EGFP. Mutational analysis confirmed critical interactions between the fluorophore and elements of the fused peptides. Solution scattering studies indicate that the Ca2+-free form of GCaMP2 is a compact, pre-docked state, suggesting a molecular basis for the relatively rapid signaling kinetics reported for this indicator. These studies provide a structural basis for the rational design of improved Ca2+-sensitive probes.

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**Interference In Coiled-coil Mediated Coupling Between Stim1 And Orai Channels**

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STIM1 and ORAI1, the two limiting components in the CRAC signalling cascade, have been reported to couple tightly upon store-depletion culminating in CRAC current activation. Based on the homology within the ORAI protein family, an analogous scenario might be assumed for ORAI2 as well as ORAI3 channels as both are activated in a similar store- and STIM1-dependent manner. A combined approach of electrophysiology and confocal Förster Resonance Energy Transfer (FRET) microscopy revealed a general mechanism in the communication of STIM1 with ORAI proteins that involved the predicted second coiled-coil motif in STIM1 C-terminus and the conserved putative coiled-coil domain in the respective ORAI C-terminus. Of the latter, a much higher coiled-coil probability is predicted for ORAI2 as well as ORAI3 than for ORAI1, compatible with our observation that a single point coiled-coil